REMARKS

Applicants are suggesting that claim 1 is amended in order to specify that the autocatalytically maturing zymogen added in step c) is an aspartic protease. It is believed that such an amendment should address the issues raised in the Advisory Action that was mailed on December 19, 2002.

In the Advisory Action, it is noted that all of the previous objections have been withdrawn with the exception of the rejection of claims 1, 4-20, 23-26, 28-30 and 41-44 under 35 USC §112, first paragraph as lacking enablement. It appears that the Examiner has two main concerns with respect to enablement. The first is the scope of the pro-peptide which can be from any aspartic protease and the second is the scope of the mature zymogen which is added in step c) of claim 1. Each of these are addressed in turn below.

With respect to the first issue, the chimeric nucleic acid sequence in the method and composition claims specifies that the pro-peptide can be from any autocatalytically maturing aspartic protease. The Examiner comments that only one aspartic protease, chymosin, is supported by a working example. Applicants maintain their position that undue experimentation would not be required by one of skill in the art in order to enable the full scope of the claims with respect to using a pro-peptide from any aspartic protease. Aspartic proteases are a group of zymogens with both a similar protein structure and mechanism of action. In particular, all aspartic proteases have the following characteristics:

- They all have an active site that contains two invariant aspartate residues (Davies, *Ann Rev Bioph Chem* 19:189-215, 1990), positioned in close geometric proximity, within a substrate cleft between bilobal N- and C-terminal domains (Foltmann, *Biol Chem Hoppe-Seyler* 369:311-314, 1988).

- They are all activated by the proteolytic removal of the N-terminal pro-peptide,usually 44-50 amino acids long, following synthesis (Foltmann, *Biol Chem Hoppe-Seyler* 369:311-314, 1988; Boel et al., *Proteins: Structure, Function, and Genetics* 1: 363-369, 1986).
- They are all inhibited by pepstatin A (Umezawa et al., *J Antibiotics* 23:259-262, 1970) and are optimally active in an acidic pH ranging from 1-5. (László, *CRC Press Inc.*, Boca Raton, Florida, USA. pp 157-182, 1989).
- They all have a catalytic mechanism which is characterized by nucleophilic attack of the scissile peptide bond by an activated water molecule and does not involve a covalent intermediate (Davies, *Ann Rev Bioph Chem* 19:189-215, 1990). At low pH one aspartate of the catalytic dyad is ionized and the second aspartate is protonated with a water molecule polarized between the aspartyl residues by hydrogen bonding. The oxygen of the water molecule engages in nucleophilic attack of the substrate carbonyl carbon of the peptide bond resulting in a tetrahedral carbon intermediate. The peptide bond is ultimately cleaved following donation of hydrogen from an aspartate to substrate nitrogen.

Therefore, aspartic proteases display the same characteristics with respect to structure, enzyme activation, and the catalytic mechanism of peptide cleavage. As a result, we submit that one of skill in the art would readily expect that a pro-peptide from any aspartic protease would be useful in the present invention.

The second issue that the Examiner raises is that undue experimentation would be required in order to determine which autocatalytically maturing zymogen could be added for cleavage of the pro-peptide. In this regard, we are suggesting that claim 1 be amended in order to specify that the zymogen added in step c) of claim 1 is an aspartic protease. Applicants have provided four examples of aspartic proteases that can cleave the chymosin pro-peptide such as chymosin (see Examples 1 and 2 of the application); red turnip beetle gut extract which comprises a mixture of proteases including aspartic proteases (see Example 3 of the application); pepsin and

Aspergillus saitoi acid protease (Sigma 2143) as described in our amendment dated September 18, 2001. Consequently, we respectfully submit that with four examples of aspartic proteases that can cleave the chymosin pro-peptide from heterologous polypeptide, it would be reasonable to predict that other aspartic proteases can also be used in this regard especially in view of the structural similarities of the aspartic proteases as described above.

With regard to the other conditions required in order to achieve cleavage, we submit that one of skill in the art could readily determine the conditions once they have selected a particular aspartic protease pro-peptide sequence and a mature form of an aspartic protease to be added to assist in the cleavage reaction. The optimum pH and other conditions required to achieve cleavage for various aspartic proteases are well known and readily available to one skill in the art.

In view of the foregoing, we submit that the claims (as amended herewith) are enabled by the specification. I look forward to our telephone interview on January 30, 2003.

Respectfully submitted,

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